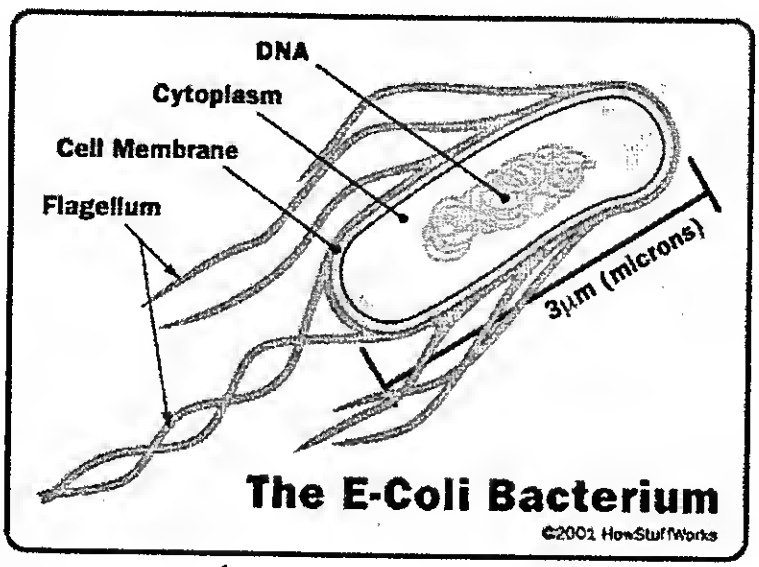
Gram –Ve Bacteria

Family

Enterobacteriaceae



أ.د/ جمال يونس

F. Enterobacteriaceae

general characters:

1. Gram negative straight rods
2. Relatively small to medium size
3. Non-sporulated
4. Some strains are capsulated.
5. Motile by lateral (peritrichous) flagella if present.
6. Facultative anaerobic
7. grow well on the surface of ordinary media and MacConkey's agar with the production of relatively large colonies.
8. Non-pigment producer except:
   - → Serratia ⟶ produce red pigment (prodigiosin)
   - → Enterobacter ⟶ yellow pigment.
9. Biochemically,
   - → Catalase ⟶ +
   - → oxidase ⟶ −
   - → active in CHo fermentation with production of acids and sometimes gases.
10. Some of them especially proteus produce swarming (spreading) on the surface of solid media.
11. Contain Common enterobacterial antigen.

Virulence Factors of Enterobacteriaceae

Factors related to bacterial cell	Factors related to bacterial products
→ Endotoxins → Capsule → Fimbriae → Invasiveness	→ Exotoxins → Haemolysin → Siderophores (chelating agents)

(A) Factors related to bacterial cell

(1) Endotoxins:

→ They are Lps (represent the outer layer of cell wall)
→ the main endotoxic principle is Lipid-A ⟶ which interfere with the complement components.
→ they are released when bacteria die and lyse (either inside or outside phagocytic cells)
→ you have to take in consideration death of bacterial cells outside phagocytic cells like complement-mediated lysis or antibiotic-mediated lysis.

→ Cause

1- Fever
2- Leukopenia followed by leukocytosis
3- hyperglycemia then hypoglycemia and lethal shock after a latent period.

② Capsule:

→ It is antiphagocytic substance where its surface is hydrophilic which repel hydrophobic surface of phagocytes

→ Interfere with binding of Abs to bacteria.

→ poor activator of complement ⟶ protect cell wall of bacteria from damaging effects of complement.

③ Fimbriae (pili, adhesins):

→ allow adhesion of bacteria to host cells with subsequent colonization ⟶ such as those in **E. coli** allow its attachment to glycoproteins found on the surface of epithelial cells of jejunum and ileum followed by colonization ⟶ So produce its pathogenicity.

④ Invasiveness:

→ ability of M.o to attack host cells.
→ Invasive strains such as **salmonella typhi** and **s. dublin** ⟶ are able to attack the host cells causing their damage ⟶ bloody diarrhea.
→ It is plasmid (genetically)-controlled.

(B) Factors related to bacterial products

(1) Exotoxins:

Enterotoxins

2 types acc. to the effect of heat:

Labile type (LT)	stable type (ST)
destroyed by heating at 60°C/30 min.	destroyed by heating at 121°C/15 min.

verotoxins (Cytotoxins, shiga-Like toxins)

→ destroy vascular endothelial cells causing bloody diarrhea.
→ they inhibit protein synthesis in host cells by interaction with 60S ribosomal subunit.

(2) Haemolysin:

→ ∝-haemolysin causes damage to the host cell membranes.

③ Siderophores (cheLating agents):

→ They are LoW moLeCuLar maSS (MM) Iron binding Compounds produced by Some bacteria ⟶ Which enabLe them to utiLize the iron required For their groWth.

→ they aid the bacteria in the Competition With host For Iron (Which is an abSoLute requirement For the groWth oF moSt bacteria).

→ Bacteria produCe Iron-reguLated outer membrane (OM) proteins (IROMPs) ⟶ reSponSibLe For the internaLization oF SiderophoreS-iron Compounds into bacteriaL CeLL.

CLaSSiFiCation:

49 genera (differentiated biochemiCaLLy)
→ the moSt important oneS:

1- G. EScherichia
2- G. SaLmoneLLa
3- G. KLebSieLLa

genus: EScherichia

E.CoLi

pathogenicity:

→ E.CoLi is naturaLLy inhabit the intestinaL tract of man, birds and animaLS.
→ It is an opportunistic organism where it turns into pathogenic state under certain Conditions.
→ Cause Food poisoning in human.

1- CattLe, sheep and goats:

- CaLves and Lambs (↓ 1 weeK oLd):
  - → CoLibaciLLosis (white SCours)
  - → CoLiseptiCaemia
  - → joint_iLL in those surviving CoLiseptiCaemia.

- aduLts:
  - → enteritis
  - → CoLiForm mastitis
  - → abortion
  - → urogenitaL infection
  - → pneumonia.

2-Equines:

Foals

→ Colibacillosis
→ Coli septicaemia
→ joint ill and Navel ill With other bacteria.

3-poultry and Rabbits:

→ Colibacillosis (Coliseptic aemia)
→ Coligranuloma (Hjarre's disease)
→ egg peritonitis
→ Omphalitis (mushy chick disease, yolk sac infection, Navel infection to embryo)
→ Synovitis, arthritis
→ Salpingitis (oviduct infection)
→ panophthalmitis (eye infection)
→ enteritis
→ tracheitis, air-sacculitis
→ Localized abscesses and Coli-enteritis in Rabbits.

Types of pathogenic E.coLi

→ there are 5 types acc.to mechanism of gastroenteritis

Entero-toxigenic E.coLi (ETEC)	Entero-invasive E.coLi (EIEC)	Entero-pathogenic E.coLi (EPEC)	Entero-aggregative E.coLi ($EAggEC$)	Entero-haemorrhagic E.coLi (EHEC)
→ produce **enterotoxins** (which may be LT or ST) → genetically-controlled (plasmid-controlled) → F-antigens are attached to the surface of intestinal epitheLial cells → For toxin production. → Cause **gastroenteritis** which is ch' by: ① Vomiting ② secretory diarrhea (traveLer's diarrhea) → SimiLar to that caused by Vibrio choLerae. → **Cause Food poisoning in man.**	→ **Invade** the intestinaL epitheLiaL cells. → pLasmid-controLLed → Cause **bLoody diarrhea** (dysentery-Like disease) SimiLar to that caused by shigeLLa species	→ Contain **adherence Factor pLasmid** → It **adheres** to the intestinaL epitheLiaL cells → this adhesion is foLLowed by inFLammation. → Cause **diarrhea in Lambs and chiLdren.**	stacked brick Like aggregative pattern of adherence is Formed due to the presence of **aggregative adherence pLasmid.**	→ produce **cytotoxins (shiga-Like toxin)** which damage the vascuLar endotheLiaL cells in the intestinaL tract causing **bLoody diarrhea in humans** SimiLar to shigeLLa dysenteriae → So, it is caLLed **haemorrhagic** CoLitis in man.

Products of E. CoLi :

① Exotoxins :

Enterotoxins

- **LT**
  - **LT_I**: affect adenyLate cycLase system Causing diarrhea, hypovoLemia, Metabolic acidosis and hyperKaLemia
  - **LT_II**: produced by strains of E. CoLi isoLated From animaLs but not From human.
- **ST**
  - **STb**: produced by strains of E. CoLi isoLated From animaLs but not From human.
  - **STa**: affect guanyLate cycLase system Causing FLuid accumuLation in intestine of suckLing mice and pigLets

Verotoxins (cytotoxins, shiga_LiKe toxins)

- **LT_1**: neutraLized by shiga antitoxin
- **LT_2**: → Not neutraLized by shiga antitoxin
  - → Causing haemorrhagic coLitis in man.
  - → Cause death in miCe
  - → induce changes in Vero CeLLs in TC

② HaemoLysins : 4 types

- **α and β**: produce cLear Lysis zone and indistinguishabLe on bLood agar pLates
- **Entero_haemoLysin**: Seen on media Containing washed sheep, rabbit or human RBCs
- **δ**: active on horse, G. pig and sheep RBCs

③ CoLiCins (antibiotic substances):

→ In a mixed culture, pathogenic E.CoLi produces CoLiCins which KiLL other bacteria through their attachment with specific receptors on the surface of these bacteria.

→ E.CoLi:

1- may produce CoLiCins (CoLiCin +ve E.CoLi)

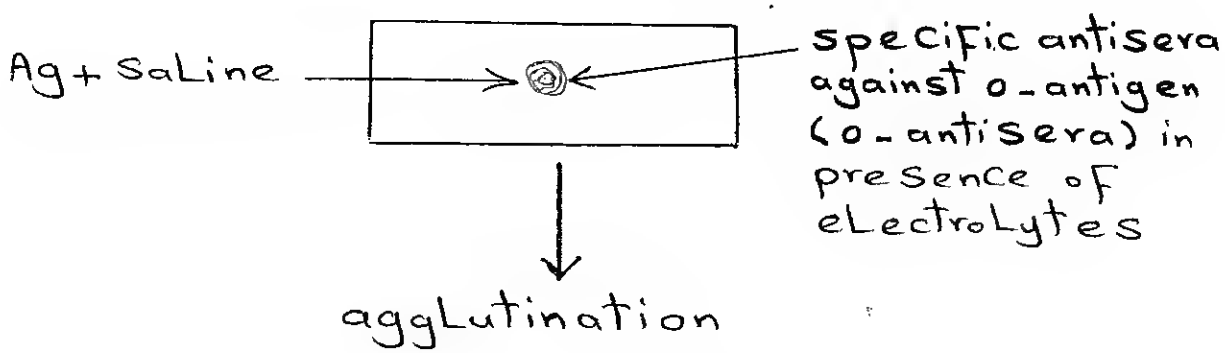
2- may not produce CoLiCins (CoLiCin -ve E.CoLi)

→ CoLiCin_V ——→ It is a ViruLenCe Factor where E.CoLi has CoLV pLasmid is more ViruLent and their ViruLenCe decrease with Loss of this pLasmid.

antigenic structure of E.coLi

Somatic(O) antigens	CapsuLar(K) antigens	FLageLLar(H) antigens	FimbriaL(F) antigens
LipopoLysaccharide (LPS) in nature (CeLL WaLL antigens)	poLysaccharide in nature (May be protein in nature such as K88 and Kgg)	protein in nature	gLycoprotein in nature
Heat_stabLe	there are 3 types acc.to the effect of heat: ① L ← Inactivated by heating at 100°C/1hr ② B ← Inactivated by heating at 100°C/1hr ③ A ← Inactivated by heating at 121°C/2.5 hrs.	Heat_LabiLe (because it is protein in nature)	Heat_LabiLe
Not destroyed by aLcohoL but destroyed by FormaLin		destroyed by aLcohoL but not destroyed by FormaLin	
Monophasic		Monophasic	
there are **181** O-antigens	There are about **74** K-antigens	There are about **57** H-antigens	
Identification of Somatic Ag is done by using O-antisera	Identification of CapsuLar Ag is done by using K-anti sera	Identification of FLageLLar Ag is done by using H-anti sera	
	retard Somatic aggLutination → to avoid this problem → make repeated subCuLture of bacteria which Lead to Loss of Capsule		→ they are adhensive structures to the CeLLs → pLasmid-ControLLed

Somatic agglutination:



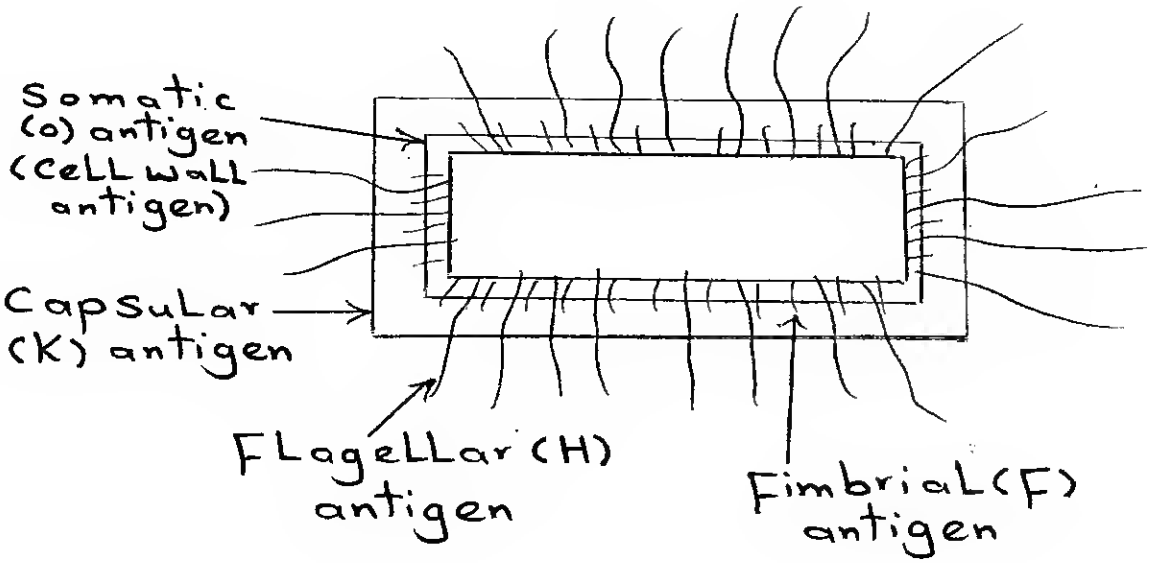
→ **preparation of O-antisera:**

1- destruction of other antigens by addition of alcohol (O-antigen not destroyed by alcohol)
2- Inoculation of O-antigen into Lab. animal ⟶ Lead to production of O-antisera

→ **Capsule retard or interfere with Somatic agglutination of E. Coli**

1- Can't destroyed by heating as it is heat-stable
2- So, to make Somatic agglutination ⟶ make repeated subculture of bacteria which Leads to Loss of Capsule

N.B: any protein is heat-Labile except:
1- staphylococcal enterotoxin
2- botulism toxin



antigenic Formula of E.coli

- O-antigens
- K-antigens
- H-antigens

→ antigenic Formula determine Serotype.

→ antigenic Formula of Some E.coli strains:

E.coli	O	:	K	:	H
	10	:	5	:	4
	45	:	ne	:	23
	117	:	98	:	4

Laboratory diagnosis:

① Isolation:

1- O_2 requirement ⟶ Facultative anaerobic
2- opt. temperature ⟶ 37°C (20-44°C)
3- opt. pH ⟶ Neutral
4- Incubation time ⟶ 1-3 days.
5- grow well on:
- → ordinary media
- → Blood agar
- → MacConkey's agar
- → Xylose-Lysine desoxycholate (XLD)
- → Eosin methylene blue agar (EMB)
- → Salmonella-Shigella agar (S-S agar)

② Identification:

a- Culture characters:

1- E. Coli is non-pigment producer

2- on MacConkey's agar:

pink colonies due to Lactose fermentation and acid production
→ Contain Neutral red indicator

3- on EMB medium:

green black colonies with a metallic sheen.

4- on XLD agar:

yellow colonies due to xylose utilization with acid production

→ Indicator : phenol red

b- Morphology:

→ stain used → Gram's stain
→ staining reaction → Gram -ve
→ shape → straight rods
→ size → relatively small to medium
→ spore → Non-sporulated
→ Capsule → Some strains develop Capsule
→ Motility → motile with peritrichous flagella

C- Biochemical identification:

Test	E.coli	Klebsiella	Salmonella
H_2S	−	−	+
urease	−	slow	−
I M Vi C	+ + − −	− − + +	− + − +

→ E. coli:

1- Ferments glucose and lactose with acid and gas production
2- Reduce nitrate.

d- Serological typing (sero-typing):

use slide agglutination test to determine the serotype through the identification of its O, K and H antigens by specific antisera.

e- demonstration of pathogenic E.coli:

E.coli may be pathogenic or non-pathogenic
→ therefore determination of pathogenic E.coli is done by studying the virulence factors of the isolated strain as:

- → Fimbrial antigens
- → enterotoxins
- → Cytotoxins

dr. Mahmoud Abdel naeem
(Bv.sc, Mv.sc, ph.D)
0100 39 12 810

genus: KLebsieLLa

species:

K. pneumoniae and others

pathogenicity:

1. pneumonia in CattLe, horses and poultry
2. Mastitis in CattLe
3. Metritis in mare
4. pneumonia and urinary tract infection in man.

Laboratory diagnosis:

1. ISoLation:

LiKe E.CoLi

2. IdentiFication:

a. Culture characters:

1- on soLid media:

CapsuLated strains appear as muCoid CoLonies varying in diameter (1 - 4 mm) ⟶ aFter repeated SubCuLture, there is tendenCy For muCoid CapSuLes to be Lost and the CuLture appear as SmaLL CoLonies and Less muCoid SimiLar to E.CoLi.

2- on blood agar: Non-haemolytic

3- on MacConKey's agar:
pink colonies due to Lactose fermentation

4- on S-S agar:
Like MacConKey's agar

5- on XLD agar:
yellow colonies due to xylose utilization

b- Morphology:

- stain used ⟶ Gram's stain
- staining reaction ⟶ Gram -ve
- shape ⟶ short thick rod shaped bacilli with rounded end (sausage shape)
- size ⟶ Medium
- Non-sporulated
- produce polysaccharide capsule which give rise to mucoid slimy colonies.
- Non-motile

c- Biochemical characteristics:

1- H_2S	urease	IMViC
-	slow	- - + +

2- gelatin Liquefaction ⟶ -ve

3- CHo Fermentation ⟶ Fermentation of glucose, Lactose, sucrose, salicin, raffinose and rhamnose with acid and gas production.

antigenic structure and serotyping:

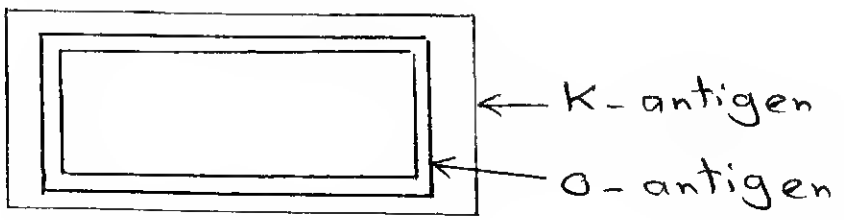
- antigenic structure is complex and consists of ⟶ 11 o-antigens
  ⟶ 80 K-antigens

- Serotyping of Klebsiella depend on K-antigens only ⟶ because:

① Capsular antigen produced in Large amount.

② because K and O antigens are thermostable ⟶ it is difficult to separate them

③ Capsular Ag retard or inhibit somatic agglutination.



genus: Salmonella

pathogenicity:

1- Salmonella is a facultative intracellular parasite.
2- Most of its serovars are potential pathogens for both man and animals.
3- Zoonotic M.o
4- Cause food poisoning in man.

animal	disease	Caused by
Cattle	1- abortion	S. dublin
	2- Enteritis and septicaemia in calves	S. dublin, S. typhimurium and S. newport
	3- Meningitis, osteomyelitis and terminal dry gangrene in calves	S. dublin
sheep and goat	1- abortion	S. abortus ovis, S. montevideo and S. dublin
	2- Enteritis and septicaemia in Lambs and Kids	S. dublin S. typhimurium
Horses	1- abortion in mares	S. abortus equi
	2- Enteritis and septicaemia in foals	S. typhimurium

poultry	1- pullorum disease (Bacillary White diarrhea)	S. pullorum
	2- Fowl typhoid (gallinurum disease)	S. gallinarum
	3- Arizona infection (arizonosis, paracolon infection)	S. arizonae
	4- avian paratyphoid (Keel disease in duckling)	other serovars of Salmonella such as S. typhimurium

→ Transmission of Salmonella in poultry:

Vertical (transovarian) transmission

S. pullorum, S. gallinarum and S. arizonae

↓

transmitted to egg yolk through transovarian infection where infected birds carry the M.o in their ovaries and testicles.

horizontal transmission

↓

through Contaminated egg shell

Zoonotic importance:

- Salmonella causes the following diseases in human:

① Typhoid Fever ⟶ Caused by S. typhi

② paratyphoid Fever ⟶ Caused by
- → S. paratyphi A
- → S. Schottmuelleri (Formerly S. paratyphi B)
- → S. Hisvchfeldi (Formerly S. paratyphi C)

③ Food poisoning ⟶ Caused by other Salmonella serovars esp. S. typhimurium and S. enteritidis.

- Salmonella is transmitted by faecal-oral route.

- Rodents (wild rat and mice):

Infected rodents excrete Salmonella in their faeces contaminating both human and animal feed as well as general environment ⟶ So, Rodents retard programmes of Salmonellosis control.

- Recovered cases from Salmonellosis become **Carrier** (subclinical excretors)

- Infected and Carrier Cases desiminate Salmonella in their Faeces Causing Contamination of Food, Water and environment.

- animal Salmonellosis has a public health importance → Where the disease in one animal may be a Source of infection to a group of animals → which is the origin of human infection Causing Food poisoning in human through Consumption of meat, milk, eggs ...

- Food handlers → Can transmit infection From human to human (Faecal-oral route)

N.B:

1- Salmonellae are protected From acidity of stomach and the acidic pH of phagosome by an acid tolerance response (ATR) gene.

2- Catalase and Superoxide dismutase protect the bacteria From intracellular Killing by phagocytes through their inhibition of oxidative Killing inside phagocytic cells.

Laboratory diagnosis:

① Isolation:

1- Cultivation on selective enrichment broth:

- Such as → Selenite F broth
  - → tetrathionate broth
  - → Rappaport Vassilidis broth

- This step is done to enhance the growth of Salmonella and inhibit the growth of other enteric bacteria.

- The inoculated tubes are incubated aerobically at 43 °C or 37 °C.
  - → With addition of Na sulphathiazole at 1.25 mg/L
  - → The incubation time → Not more than 18 - 20 hrs to suppress the growth of proteus spp.

2- plating on selective differential solid media:

- Such as → MacConkey's agar
  - → Salmonella-shigella agar (S-S agar)
  - → Desoxycholate citrate agar (DCA)
  - → brilliant green agar
  - → xylose lysine desoxycholate agar (XLD)

- The inoculated plates are incubated aerobically at 37°C for 1-3 days.
- Suspected colonies of salmonella are picked up and subcultured onto the surface of selective differential solid media to get a pure culture.

Q: How can you obtain a pure culture of an organism suspected to be salmonellae from intestinal contents (2006)

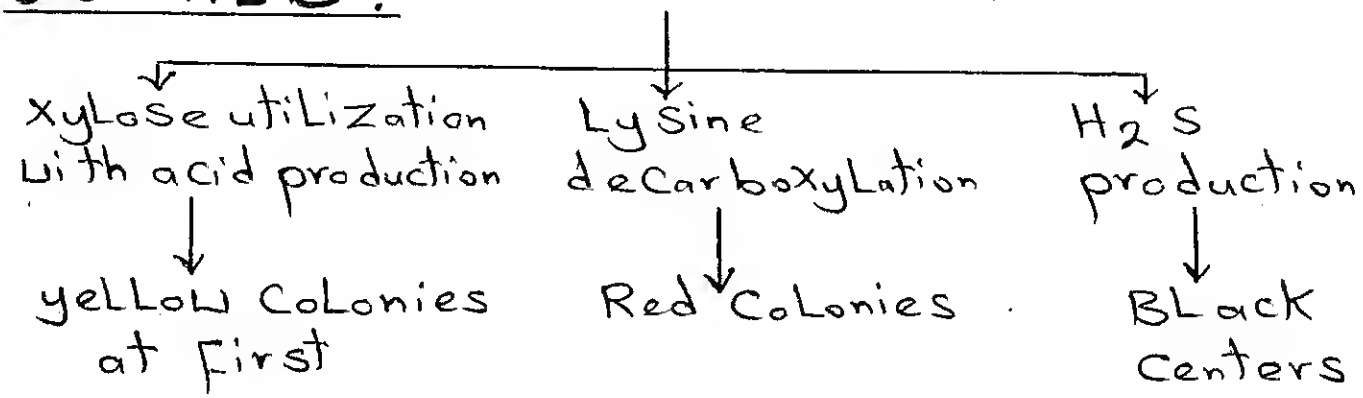
② Identification:

a- Culture characters:

- **on nutrient agar:**
  Smooth colonies

- **on MacConkey's agar:**
  salmonellae produce colorless colonies because they are non-lactose fermenter except S. arizonae (Late lactose fermenter, after 3 days)

- **on S-S agar:** as MacConkey's agar
- **on DCA:** as MacConkey's agar
- **on brilliant green agar:** pink colonies
- **on XLD:**



→ Indicator ⟶ phenol red indicator

b- Morphology:

- **stain used** ⟶ Gram's stain
- **staining reaction** ⟶ Gram -ve
- **shape** ⟶ rods
- **size** ⟶ small to medium
- **spore** ⟶ Non-sporulated
- **capsule** ⟶ may be capsulated
- **motility** ⟶ motile with peritrichous flagella except S. pullorum and S. gallinarum (Non-motile)

c- biochemical Identification:

H_2S	urease	I	M	Vi	C
+	−	−	+	−	+

- gelatin Liquefaction test ⟶ -Ve
- Reduce Nitrate to Nitrite
- do not ferment sucrose, salicin and Lactose except S. arizonae which is Late Lactose fermenter.
- decarboxylase Lysine, arginine and ornithine.

d- Serological identification:

1- agglutination test:

Slide (rapid) agglutination test

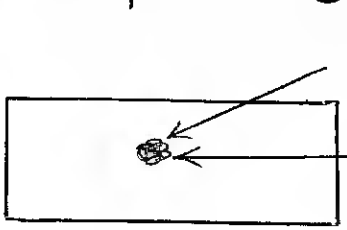
→ made by using polyvalent and monovalent O and H Salmonella antisera to determine the serovar of isolated strain.

→ must be confirmed by tube agglutination test.

Tube (slow) agglutination test

It is a confirmatory test for the slide test and used for determining the titre of infection.

- **In poultry:**



+ve result
visible clumping of colored antigen.
→ this test is called whole blood plate test
→ used in field.

2- other serological tests → ELISA, FAT, CFT

e- Molecular techniques:
- → DNA probes
- → PCR

F- phage typing:
It is very important in epidemiology and based on the sensitivity of a particular isolate to a series of bacteriophages at appropriate dilutions.